(d) said *ssaM* gene consists of SEQ ID NO: 7 or 30, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 7 or 30 under stringent conditions, or a full length Ssa-encoding nucleotide sequence that has 95% sequence identity to SEQ ID NO: 7 or 30;

and further wherein stringent hybridization conditions comprise hybridization in 50% formamide with washing at 65°C.

REMARKS

I. The Subject Matter of the Claims

In general, the subject matter of the claims relates to vaccine compositions comprising mutations in two or more genes of the secretion system apparatus (*ssa*) family of genes located in the Salmonella Pathogenicity Island 2 (SPI2) region of *Salmonella* bacteria.

II. Amendment

IN THE SPECIFICATION

The specification was amended at page 10, line 3 to include nucleic acid hybridization conditions from the Holden et al. publication, which was incorporated by reference in the application as filed.

IN THE CLAIMS

Support for the amendment to claim 5 to recite stringent conditions is found at page 10, lines 2-4, of the specification as amended herein.

Pursuant to 37 C.F.R. § 1.121, a marked-up version of the amendment to the specification and claims contained herein is attached as Appendix A. For the convenience of the Examiner, a copy of the claims being examined as they would appear upon entry of the present amendment is attached as Appendix B.

The amendment includes no new matter.

III. Restriction Requirement

Applicants renew the objection to the restriction requirement on the basis that restriction is improper for the presently pending claims.

The Examiner has restricted Applicants to a specific gene and to a specific sequence thereof that is to be mutated in the vaccine composition set forth in the invention. As stated in Section I above, and as recited explicitly in claim 1, the vaccine composition comprises a first attenuated, non-reverting mutant *Salmonella* bacterium in which two or more genes within the SPI2 region have been inactivated. (Emphasis added.) A requirement to elect one gene is inconsistent with the nature of the claimed subject matter, which involves mutations in at least two genes. *See* M.P.E.P. § 803.04. The present restriction requirement does not address whether more than one independent and distinct invention is claimed in the application. Rather, the restriction requirement effectively redefines Applicants' invention as two assertedly independent and distinct inventions. The redefined "inventions" are not consistent with Applicants' definition of the invention in the claims as originally filed. There is no basis in the law or in the Commissioner's guidance provided in the M.P.E.P. for such a redefinition of Applicants' invention.

For the foregoing reason, Applicants submit that the restriction requirement imposed by the Examiner to elect one specific gene and one specific sequence is improper and the restriction requirement should be withdrawn.

IV. Patentability Arguments

A. The Enablement Rejection of Claims 1-4, 8 and 11-14 under 35 U.S.C. § 112, First Paragraph, May Properly Be Withdrawn

The Examiner rejected claims 1-4, 8, and 11-14 under 35 U.S.C. § 112, first paragraph, for assertedly not enabling a vaccine composition comprising an immunologically protective amount of an attenuated *Salmonella* bacterium in which two or more genes within the SPI2 region of the *Salmonella* genome have been inactivated. The Examiner further contends that undue experimentation would be required to determine which genes would need to be inactivated to produce an attenuated non-reverting strain. In support of the position, the Examiner relies on Linde et al., Vaccine 8:278-282 (1990) (hereinafter, "Linde") for the proposition that multiple gene disruptions have an unpredictable effect on virulence and immunogenicity, and Hensel et al., Mol. Micro. 24:155-167 (1997) (hereinafter, "Hensel") to establish that *Salmonella* strains having mutations in *ssaC* and *ssrA* exhibit wild-type replication levels in macrophages. Relying on Linde and Hensel, the Examiner concluded that identification of the claimed subject matter would require undue

experimentation because it was unpredictable and, thus, the claims were not enabled. The Applicants respectfully traverse.

Applicants submit that the Examiner's reliance on Linde is misplaced. Linde reports that some *Salmonella* strains containing two or more attenuating mutations, which do not involve mutations in at least two SPI2 genes as recited in the pending claims, may be over-attenuated for less susceptible host species. (*See* abstract.) Over-attenuated, however, does not mean inoperable. As noted in Linde, a less susceptible host species such as a chick required three inoculations to achieve the same "good results" achieved in mice with a single inoculation. (*See* Linde at page 278.) One of ordinary skill in the art would have been aware that host species can differ in their susceptibility to any immunogen such as a vaccine and that nothing more than routine optimization would be required to determine the dose and administration schedule. Whether a given vaccine composition produces a protective immune response after one or twenty inoculations, for example, is irrelevant to the patentability of that vaccine composition.

Applicants respectfully submit that Hensel, the other reference upon which the Examiner relied, was misconstrued. Hensel reported a functional analysis of some SPI2 genes using *in vitro* assays (serum resistance assays, complement resistance assays, and invasion and replication assays using macrophage-like RAW 264.7 cells). In the passage at page 264 of Hensel upon which the Examiner apparently relied, Hensel states: "However, strains carrying mutations in *ssaC* and *ssrA* (corresponding to *spiA* and *spiR*, respectively; Ochman *et al.*, 1996) have wild-type levels of replication in RAW 264.7 macrophages (data not shown)." Applicants note that the quoted passage of Hensel refers to the plural strains, and it makes no sense for Hensel to have used the plural to indicate that multiple copies of the very same strain (i.e., strains having identical genotypes) had been constructed. The only reasonable interpretation of the quoted passage is that Hensel was referring collectively to a first strain bearing a *ssaC* mutation and a second strain bearing a *ssrA* mutation. The collective reference to these strains resulted from their common property of replicating at wild-type levels *in vitro* in RAW 264.7 cells. Moreover, that interpretation is consistent with the remaining disclosure of Hensel. At page 264, Hensel notes that the bacterial strains used in the reported study are shown in Table 5. Inspection of Table 5 reveals a list of 13 *S. typhimurium* strains, two wild-type strains and 11 single mutant strains. Not a single double-mutant strain is listed. Thus, Hensel does not disclose or suggest a *Salmonella* strain containing mutations in at least two SPI2 genes that exhibits wild-type levels of replication in

RAW 264.7 cells in *in vitro* culture. Hensel, therefore, does not disclose or suggest a *Salmonella* strain containing mutations in at least two SPI2 genes that exhibits a lack of attenuation *in vivo*.

In contrast to Linde and Hensel, the present specification discloses working examples illustrating two species (*typhimurium* and *dublin*) of *Salmonella*. Moreover, the working examples illustrate *Salmonella* containing mutations in at least two SPI2 genes and, for each such example, useful levels of attenuation are disclosed. For example, in Tables 8, 9, 10, and 11 (pages 36-38), the specification discloses results wherein the attenuated *Salmonella* vaccine composition administered to animals comprises mutations in at least two different SPI2 genes. Tables 8 and 9 disclose that vaccination of cattle with a *ssaC/ssaT S. dublin* double mutant decreased symptoms of infection upon exposure to wild-type *S. dublin*. Additionally, Table 9 discloses that a combination of a *S. typhimurium* double mutant (*ssaC/ssaT*) and a *S. dublin* double mutant (*ssaC/ssaT*) was more effective than either vaccine composition alone. For each of these attenuated vaccine compositions, the specification provides complete instructions for preparing and using the compositions.

For the foregoing reasons, Applicants respectfully submit that reliance on Linde and Hensel was misplaced and a *prima facie* case of non-enablement has not been established for any of the rejected claims. Moreover, Applicants submit that the application as filed provides an enabling disclosure commensurate in scope to each of the pending claims. Accordingly, the rejection of claims 1-4, 8, and 11-14 under 35 U.S.C. § 112, first paragraph, for lack of enablement has been overcome and the rejection may properly be withdrawn.

B. The Written Description Rejection of Claim 5 under 35 U.S.C. § 112, First Paragraph, May Properly Be Withdrawn

The Examiner rejected claim 5 under 35 U.S.C. § 112, first paragraph, for assertedly containing subject matter that was not described in the specification in such a way as to reasonably convey that the inventor had possession of the claimed subject matter. The Examiner stated that claim 5 recites a polynucleotide segment which has 95% sequence homology to SEQ ID NO: 1 and asserted that the specification and claims do not indicate what distinguishing attributes are shared by members of the genus. The Examiner further asserted that Applicants have not described a function of SEQ ID NO: 1 which describes the genus of *ssa* genes. The Applicants respectfully disagree.

Applicants thank the Examiner for directing them to the revised interim written description guidelines and cite to Example 14 at pages 53-55 therein. In that Example, the claim recites "[a] protein having SEQ ID NO: 3 and variants thereof that are at least 95% identical to SEQ ID NO: 3 and catalyze the reaction of A → B." The analysis accompanying this claim acknowledges that procedures for making variants that have 95% identity and retain activity are routine in the art. The conclusion drawn from the analysis is that this claim satisfies the written description requirement of 35 U.S.C. § 112, first paragraph.

In the instant case, claim 5 is drawn to a vaccine composition comprising an attenuated, non-reverting *Salmonella* mutant bacterium wherein at least two genes selected from the group of *ssa* genes (*ssaT, ssaJ, ssaC* and *ssaM*) have been inactivated and each exhibits one of the following: (1) one of two expressly disclosed sequences, (2) hybridizability under specified stringent conditions to the non-coding complement of a polynucleotide having one of the specified sequences, or (3) a sequence having 95% identity to one of the specified sequences. As in Example 14 of the interim written description guidelines, the claimed subject matter is drawn, in relevant part, to nucleic acids of expressly disclosed sequences that have the "activity" of inactivity (i.e., a defective secretion system apparatus) and to polynucleotides having sequences that are 95% identical to the sequences of such nucleic acids and that share the "activity" of inactivity. Claim 5 as amended herein recites an "Ssa-encoding" nucleotide sequence that has 95% sequence identity to the expressly recited sequences. Thus, the functional inactivity is an impaired secretion system apparatus associated with the type III secretion system of *Salmonella*. Therefore, claim 5 as amended is analogous to the claim of Example 14 of the interim written description guidelines in being drawn to a nucleic acid having an expressly recited sequence and a particular activity (i.e., inactivation of the secretion system apparatus) and polynucleotides having sequences that are 95% identical thereto and that share the activity of an inactivated secretion system apparatus. For these reasons, Applicants submit that the rejection of claim 5 under 35 U.S.C. § 112, first paragraph, for lack of written description has been overcome and should be withdrawn.

C. The Rejection of Claim 5 under 35 U.S.C. § 112, Second Paragraph, May Properly Be Withdrawn

The Examiner rejected claim 5 under 35 U.S.C. § 112, second paragraph, as being indefinite in the recitation of "stringent conditions." The Applicants submit that the amendment to claim 5 to expressly recite the stringent conditions renders moot the instant

rejection of claim 5. Accordingly, the rejection of claim 5 under 35 U.S.C. § 112, second paragraph, may properly be withdrawn.

D. The Rejection of Claims 1, 3-4, 8, 10, and 12-13 under 35 U.S.C. § 102(b), May Properly Be Withdrawn

Claims 1, 3-4, 8, 10, and 12-13 were rejected under 35 U.S.C. § 102(b), as assertedly being anticipated by the disclosure of Holden. In support of the rejection, the Examiner stated that Holden discloses multiple transposon insertions into the type III secretion system of *Salmonella typhimurium* and relies on *In re Best*, 562 F.2d 1252, 195 U.S.P.Q. 430 (C.C.P.A. 1977) to shift the burden to Applicants "to show an unobvious distinction" between Holden and the claimed invention.

In response, Applicants submit that the reliance on *Best* is misplaced and the Examiner has failed to establish a *prima facie* case of anticipation of the subject matter of any of the rejected claims under § 102(b) over Holden. *Best* is informative "[w]here, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes . . . ." *Best*, 562 F.2d at 1255. In the present case, the claimed vaccine compositions and Holden's products are not identical or substantially identical, nor are they produced by identical or substantially identical processes.

The rejected claims are drawn to vaccine composition comprising a first attenuated, non-reverting mutant *Salmonella* bacterium in which two or more genes within the SPI2 region have been inactivated. Holden discloses methods for attenuating a bacterium using transposon mutagenesis (STM), and demonstrates mutating a strain of *Salmonella* in which the attenuated bacterium specifically contains a single transposon insertion. Holden states that the transposon site is designed to ensure that the site occurs only once in 2×10^{11} molecules (Holden, column 25, lines 66-67), ensuring specific integration events which happen essentially once per microorganism. Example 1 of Holden explicitly states that "in each case the exconjugant had arisen as a result of a single integration of the transposon into a different site of the bacterial genome" (Column 26, lines 31-34). Additionally, claim 1 of Holden recites a plurality of microorganisms, each of which is independently mutated by the insertional inactivation of *a* gene so that each mutant contains a different marker sequence. In contrast, the claimed subject matter is drawn to a composition comprising an attenuated, non-reverting mutant *Salmonella* bacterium in which two or more genes are inactivated.

Furthermore, Holden does not suggest, and cannot be interpreted to suggest, a bacterium containing more than one mutation. Holden discloses a subtractive screen of mutated bacteria designed to identify genes or markers associated with reduced adaptability to a given environment (*see* abstract). Holden teaches construction of a library of mutant bacterial cells, each cell containing a single mutation somewhere in the bacterial genome, and the introduction of a sample of that library into a particular environment. By subtracting members of the library that survive in a particular environment, Holden teaches identification of library members that have reduced adaptability in that environment. Holden does not teach more than one mutation per cell, and the analysis underlying Holden's method would be confounded if multiple mutations were permitted. For example, if a given cell had five insertionally inactivated genes and that cell failed to survive in a particular environment, Holden could not determine which of the five genes or markers was responsible for the reduced adaptability. Thus, Holden neither discloses, nor suggests, the presently claimed subject matter drawn to vaccine compositions comprising a mutant bacterium wherein the bacterium contains at least two mutated genes. Because Holden fails to disclose, expressly or inherently, at least two mutated genes, Holden fails to anticipate the subject matter of any of the pending claims under § 102(b).

Applicants respectfully submit that reliance on *In re* Best, 562 F.2d 1252 (C.C.P.A. 1977) was misplaced and a *prima facie* case of anticipation of any of the rejected claims under 35 U.S.C. § 102(b) over Holden has not been established. Further, Applicants submit that even if the burden were properly shifted, Applicants have established that Holden did not, and logically could not, have disclosed each element of any of the pending claims. Accordingly, Holden does not anticipate any of the rejected claims. For these reasons, Applicants submit that the rejection of claims 1, 3-4, 8, 10, and 12-13 under 35 U.S.C. § 102(b) over Holden has been overcome and the rejection should be withdrawn.

E. The Objection To Claims 6, 7, And 9 Is Moot And Should Be Withdrawn

Claims 6, 7, and 9 were subject to objection as being dependent on a rejected base claim, i.e., claim 5. As established above, claim 5 as amended is allowable. Accordingly the objection to claims 6, 7, and 9, dependent on claim 5, has been rendered moot and may properly be withdrawn.

V. Conclusion

In view of the amendment and remarks made herein, Applicants respectfully submit that claims 1-14 are in condition for allowance and respectfully request expedited notification of same.

Respectfully submitted,

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APPENDIX A

VERSION MARKED TO SHOW CHANGES

IN THE SPECIFICATION

The nucleotide sequence of *ssaT* from *S. dublin* is set forth in SEQ ID NO: 1. The nucleotide sequence of *ssaT* from *S. typhimurium* is set forth in SEQ ID NO: 2. As used herein, "*ssaT*" includes SEQ ID NOS: 1, 2 and other *Salmonella* species equivalents thereof, e.g., full length *Salmonella* nucleotide sequences that hybridize to the non coding complement of SEQ ID NO: 1 or 2 under stringent conditions, wherein stringent conditions comprise hybridization in 50% formamide with washing at 65°C (*e.g.*, as described in Figure 4 of Shea et al., Proc. Nat'l. Acad. Sci. USA, 93:2593-2597 (1996), incorporated herein by reference), and full length *Salmonella* nucleotide sequences that have 90% sequence identity to SEQ ID NO: 1 or 2. *Salmonella* species equivalents can be easily identified by those of ordinary skill in the art and also include nucleotide sequences with, e.g. 90%, 95%, 98% and 99% identity to SEQ ID NO: 1 or 2.

IN THE CLAIMS

Please amend claim 5 as follows:

5. (Amended) The vaccine composition of claim 1 wherein the genes are selected from the group consisting of *ssaT, ssaJ, ssaC* and *ssaM*, and wherein:

(a) said *ssaT* gene consists of SEQ ID NO: 1 or 2, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 1 or 2 under stringent conditions, or a full length Ssa-encoding [*Salmonella*] nucleotide sequence that has 95% sequence identity to SEQ ID NO: 1 or 2;

(b) said *ssaJ* gene consists of SEQ ID NO: 3 or 4, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 3 or 4 under stringent conditions, or a full length Ssa-encoding [*Salmonella*] nucleotide sequence that has 95% sequence identity to SEQ ID NO: 3 or 4;

(c) said *ssaC* gene consists of SEQ ID NO: 5 or 6, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 5 or 6 under stringent conditions, or a full length Ssa-encoding [*Salmonella*] nucleotide sequence that has 95% sequence identity to SEQ ID NO: 5 or 6; and

(d) said *ssaM* gene consists of SEQ ID NO: 7 or 30, or a full length nucleotide sequence that hybridizes to the non coding complement of SEQ ID NO: 7 or 30 under stringent conditions, or a full length Ssa-encoding [*Salmonella*] nucleotide sequence that has 95% sequence identity to SEQ ID NO: 7 or 30;

and further wherein stringent hybridization conditions comprise hybridization in 50% formamide with washing at 65°C.